

Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy

Volume 12

Article 26

January 2010

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Masayuki Sakakibara

Ehime University

Aya Watanabe

Ehime University

Masahiro Inoue

Ehime University

Sakae Sano

Ehime University

Toshikazu Kaise

Tokyo University of Pharmacy and Life Science

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Recommended Citation

Sakakibara, Masayuki; Watanabe, Aya; Inoue, Masahiro; Sano, Sakae; and Kaise, Toshikazu (2010) "Phytoextraction And Phytovolatilization Of Arsenic From As-Contaminated Soils By *Pteris vittata*," *Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy*: Vol. 12 , Article 26.

Available at: <https://scholarworks.umass.edu/soilsproceedings/vol12/iss1/26>

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Chapter 25

PHYTOEXTRACTION AND PHYTOVOLATILI-ZATION OF ARSENIC FROM AS-CONTAMI-NATED SOILS BY *PTERIS VITTATA*

Masayuki Sakakibara¹, Aya Watanabe¹, Sakae Sano², Masahiro Inoue¹, and Toshikazu Kaise³

¹Graduate School of Sciences and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama, 790-8577 Japan; ²Earth Science Laboratory, Faculty of Education, Ehime University, ³Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji 192-0392, Japan

Abstract: Phytoremediation of metal contaminated soil and water is a low cost method of remediation, which allows some extracted metals to be recycled for economic use. The use of phytoextraction and phytovolatilization of metals by plants offers a viable remediation on commercial projects. Arsenic is of great environmental concern due to its extensive contamination and toxicity. Ma *et al.* (2001) recently discovered the first known and extremely efficient arsenic hyperaccumulating plant, *Pteris vittata*. If we can use phytovolatilization for As-contaminated soils, it could minimize the production of As-laden plant material and the duration of remediation. Several greenhouse experiments conducted by us have shown that the material balance of As between uptake by *P. vittata* and reduction from As-polluted soils has not been maintained. Vapor samples were collected to determine volatilization of arsenic compounds from fronds of *P. vittata* grown in As-polluted soil. A low-density polyethylene bottle was placed over a stem and sealed at the open bottleneck with sealant and tape to produce a tight seal around the stem and chambers for a period of 2–7 days. Concentration of arsenic in trap samples was measured by ICP-MS and speciation of arsenic was analyzed using HPLC/ICP-MS system. Concentrations in collected water samples (ca. 0.6–5.9ml) reached 10.7–30.8 µg-As/L (2–7 days). Percentages of arsenic components in one sample were 37% for arsenite and 63% for arsenate. Our results suggest that *P. vittata* is a plant species that is effective at volatilizing As; it removed about 90% of the total uptake of As from As-contaminated soils in the greenhouse, where the environment was similar to the subtropics. However, if a large amount of arsenic had been released from the contaminated site into the atmosphere by the fern, the process may have caused a secondary As-contamination to the surrounding environments.

Key words: Arsenic, *Pteris vittata*, phytoextraction, volatilization, arsenite, arsenate, greenhouse experiment

1. INTRODUCTION

Some metal contaminants such as As, Hg, and Se also exist as gaseous species in the environment. In the recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them into gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial phytoremediation technology. Since Hg and Se are toxic (Wilber, 1980; Suszcynsky and Shann, 1995) there is an uncertainty whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Se phytovolatilization is the process by which gaseous Se is produced from inorganic or organic Se compounds (Lewis *et al.* 1966; Terry *et al.* 1992; Bañuelos *et al.*, 1993a, b; McGrath, 1998). Furthermore, the volatilization is particularly attractive for the phytoremediation of Se-contaminated environment because this element is a serious problem in many parts of the world with Se-rich soil (Brooks, 1998). Moreover, in recent years, there has been a considerable effort to insert bacterial Hg ion reductase genes into plants for Hg

phytovolatilization (Rugh *et al.*, 1996; Heaton *et al.*, 1998; Rugh *et al.*, 1998; Bizily *et al.*, 1999). On the other hand, there have been no efforts to genetically engineer plants that volatilize As. The most efficacious remediation of arsenic has been suggested where plants extract arsenic from soil and hyperaccumulate it aboveground in fronds and stems.

Pollution is a worldwide problem and there is tremendous demand for the cleanup of As-contaminated soil and water. Some plant species growing on As-contaminated soils are As tolerant and accumulate very high concentrations of As (As accumulators). An arsenic hyperaccumulator, *Pteris vittata* L. (Chinese brake), was first discovered in China by means of a field survey and greenhouse cultivation. The plant's abilities to produce large quantities of root exudates (to solubilize soil arsenic), effectively translocate arsenic to the fronds (up to 95%), reduce arsenic from arsenate-As(V) to arsenite-As(III) (up to 100% arsenite) in the fronds, and keep high concentration of P in the roots have contributed to its capability to hyperaccumulate arsenic, making it a prime candidate for use in phytoremediation of As-contaminated sites (Ma *et al.*, 2001).

The objective of this study was to determine *P. vittata*'s ability to metabolize As into volatile As forms (phytovolatilization) along with the application of phytoremediation for the cleanup of As-contaminated environments.

2. MATERIAL AND METHODS

2.1 Greenhouse experiment

The As-contaminated soil used in this experiment was collected from a deposit site of neutralized acid mine drainage in Japan. It accumulated up to $6,540 \pm 380$ mg As/kg-dry weight (DW).

One healthy fern with 6–7 fronds, about six months old, was planted in each pot containing about 12 kg of soil. The plants were grown for 18 months in a greenhouse where the average temperature varied from 25 (night) to 45°C (day) in summer and 10 (night) and 25°C (day) in winter. The concentration of As in the fronds ranged from 3,830 to 11,020 mg/kg-DW.

Water vapor samples were collected to determine volatilization of arsenic compounds from leaves of the fern to the atmosphere. A low-density polyethylene bottle (20L) was placed over a stem and sealed at the open bottleneck with sealant and tape to produce a tight seal around the stem and chambers during a 2–7 day period (Fig. 1). The bottles were fixed in the same location during sample collection.

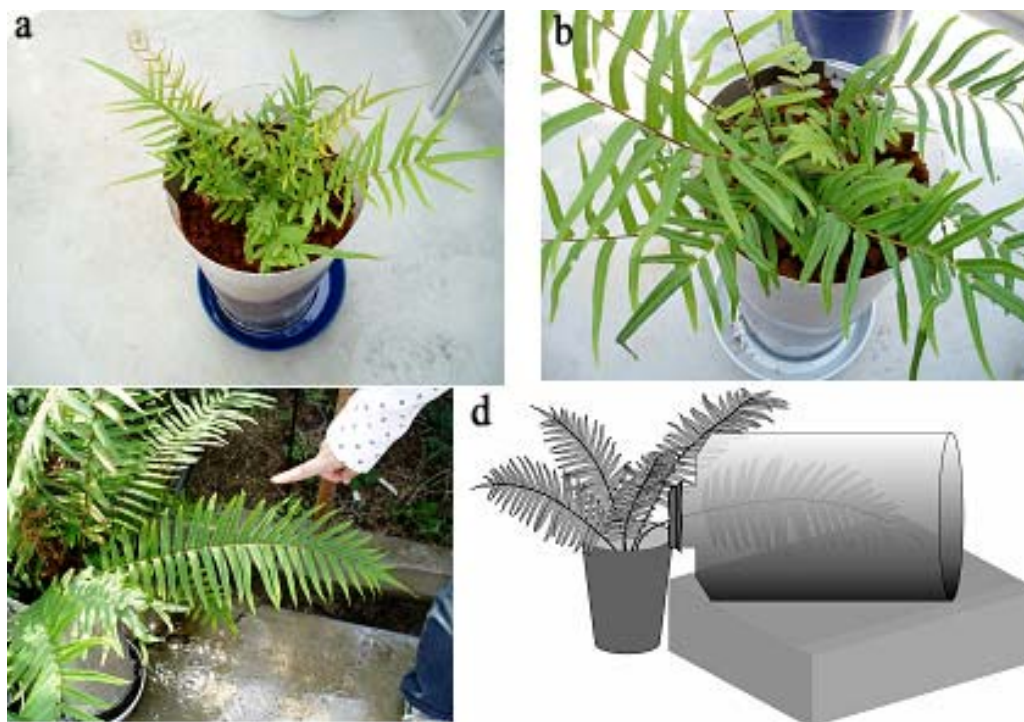


Figure 1. *Pteris vittata* for the experiment of volatilization of arsenic. a. the transplanted fern in the As-contaminated soil, b. the fern growing up, c. the frond for the volatilizing experiment, d. experiment for collecting vapor from the fern.

2.2 Analytical methods

The speciation and quantification of As were accomplished by using HPLC to separate the species and ICP-MS to detect them. Detection of arsenic species was accomplished with an ELAN® DRC-e ICP-MS (PerkinElmer SCIEX) at Tokyo University of Pharmacy and Life Science (Kamidate *et al.*, 2000). Total arsenic concentrations were also measured without speciation, using conventional nebulization into the ICP-MS at Ehime University (Sano *et al.*, 2005).

3. RESULTS

Three volatile water samples from the fern were collected for chemical analysis by ICP-MS. As-concentrations in the collected water samples were 0.74 µg-As/L in 0.6 cc (2 days), 16.1 µg-As/L in 5.9 cc (7 days), and 30.8 µg-As/L in 4.1 cc (7 days). As-concentrations of blank were 0.15 µg-As/L in 30 cc (7 days). The result of the experiment revealed that *P. vittata* is a plant species that releases As from the fronds.

Chromatographical analysis using HPLC-ICP-MS for arsenic of volatilized water detected only arsenite and arsenate. Chromatograms are presented in Fig. 2, showing that good separations were achieved for the As species. Percentages of arsenic components in one sample were 37% for arsenite and 63% for arsenate.

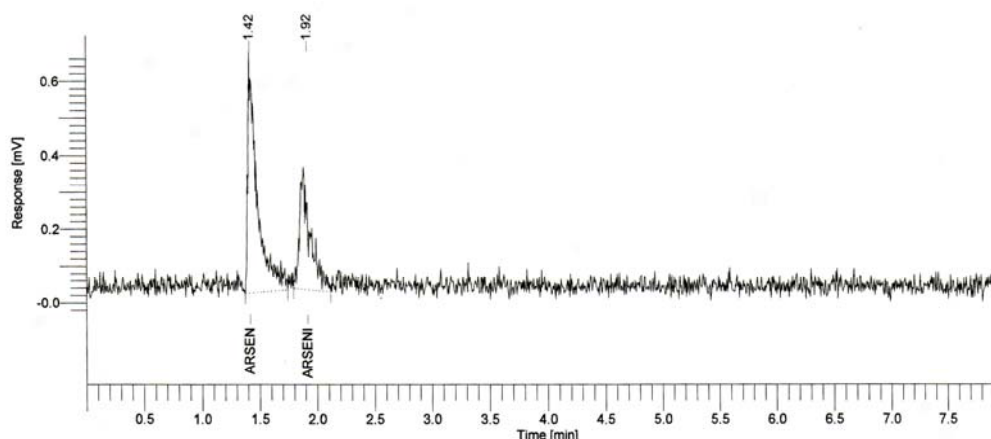


Figure 2. Chromatograms (HPLC-ICP-MS) of vapor collected from *Pteris vittata* for As speciation

4. DISCUSSION

For phytoextraction, the total amount of arsenic accumulation by the fern should be considered because it takes into account both biomass production and As concentration, measuring the potential effectiveness of a plant for phytoextraction. The amount of arsenic found in the plant tissue was well explained by the decrease in total soil arsenic after growing *P. vittata* (Tu *et al.*, 2002). Moreover, the most striking feature associated with the As hyperaccumulation by *P. vittata* lies in the exceedingly efficient transport from roots to shoots. In the depletion experiments (8 h) by Wang *et al.* (2002), between 50% and 78% of As taken up had already been transported from the culture to the fronds.

On the other hand, only 15–20% of the total As removed from the soil can be accounted for that accumulated by the fern in our recent experiments (Sakakibara *et al.*, 2004a; Watanabe *et al.*, 2005; and unpublished data).

Speciation in plant tissues by HPLC-ICP-MS is conducted on methanol/water extracts, including previous studies of As-hyperaccumulating ferns, where only arsenate and arsenite have been detected (Ma *et al.*, 2001; Francesconi *et al.*, 2002). A few studies have reported that a reduction of arsenic, arsenate ion (As^{5+}) to arsenite ion (As^{3+}), occurred in the plant body (Ma *et al.*, 2001; Lombi *et al.*, 2002; Wang *et al.*, 2002; Huang *et al.*, 2004). Wang *et al.* (2002) concluded that arsenate was taken up by *P. vittata* via the phosphate transporters, reduced to arsenite, and then sequestered in the fronds primarily as As(III). Huang *et al.* (2004) reported that the As in the fern were mainly coordinated with oxygen in the reduced state, As (III), and the reduction of As(V) occurred in the root after it was taken up, by using synchrotron radiation extended X-ray absorption fine structure (SR EXAFS). Based on our experiments, *P. vittata* accumulated large amounts of inorganic As compounds, mainly arsenite and arsenate, in their tissues and released large amounts of As from the fronds. *P. vittata* is promising for the phytoremediation of As-contaminated soil because of its remarkable ability to phytoextract As and release the accumulated As in the fronds. However, the process of volatilization of arsenic from *Pteris vittata* remains unknown.

P. vittata may release their secretions including arsenic compounds, through secretory glands at the edge of the fronds. Hokura *et al.* (2006) revealed that the arsenic distributions of the fern were successfully measured by the μ -SR-XRF analysis, and As(V) in the culture medium was reduced to As(III) in the tissue after absorption. The As contents are characteristically high at the edge of the frond concentrating secretory glands, based on the As imaging of XRF. However, if large amount of arsenic would have been removed from the contaminated site into the atmosphere by the fern, then the process would have caused a secondary As-contamination to the surrounding environment.

5. CONCLUSION

It was determined that vapor released from the frond of *P. vittata* included arsenic compounds, arsenite and arsenate. The results suggest that *P. vittata* effectively volatilizes As; it removed a maximum ratio of 90% of the total uptake of As from As-contaminated soils in greenhouse, where the environment was similar to the subtropics. However, if a large amount of arsenic had been released from the contaminated site into the atmosphere by the fern, the process may have caused a secondary As-contamination to the surrounding environment.

ACKNOWLEDGEMENTS

We would like to thank the Grant for Research and Development Assistance of Ehime University for their support. We also thank Dr. Kuramoto for his assistance in ICP-MS analyses and Mr. Toshihito Kondo in FUJITA Co. Ltd. for providing the seedlings of *P. vittata*.

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